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PULMONARY ANTIOXIDANTS

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16. ABSTRACT

One of the most vital of the cellular defenses against pollution is an 'antioxidant armamentarium' which consists of oxidant scavenging molecules such as vitamin E, glutathione, vitamin C and uric acid as well as a number of enzymes (superoxide dismutase, semidehydroascorbate reductase, catalase, GSH synthetase, GSH peroxidase, GSH reductase, and GSH transferase) and appears to function in keeping oxidant forces under control. Pollutants can upset the oxidant/antioxidant balance of cells by inhibiting vital enzymes, by reacting with oxidant scavengers, or by forming free radical intermediates which initiate uncontrolled tissue reactions with molecular oxygen. This book chapter reviews possible interactions between pollutants and the oxidant/antioxidant balance. EPA's need for this type of information falls into three categories. First, there is a need for understanding differences between the tissue sensitivity of animals and humans for purposes of quantitative extrapolation as outlined in the previous peer review documents. Second, the Clean Air Act mandates EPA to protect even the weakest of the human population from the effects of pollution. An understanding of tissue defenses and their alterations is essential to the accomplishment of this goal. Third, changes in antioxidant/oxidant status of tissues brought on by pollutants may themselves serve as sensitive toxicity endpoints as further understanding of their adversity is gained through research.

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Biological membranes maintain the structural and functional organization of living cells. In effect, they define the cell, isolate it from its environment, and sub-divide it into subcellular compartments (e.g. nucleus, mitochondria, endoplasmic reticulum, lysosomes, etc.). The plasma membrane functions to regulate transport of substances into and out of the cell. Compartmental membranes, likewise, regulate the internal milieu of the subcellular compartments. Biomembranes also are sites of attachment of enzymes and proteins involved in transmembrane transport and receptor recognition functions.

Biomembranes are comprised primarily of phospholipids and proteins. The phospholipids are rich in polyunsaturated fatty acids (PUFA) which are highly susceptible to oxidative damage (Plaa and Witschi, 1976). For example, it has been demonstrated that ozone (O_3) and nitrogen dioxide (NO_2), two of the principal oxidants in urban ambient air and photochemical smog, can damage membrane lipids via lipid peroxidation (e.g. Plaa and Witschi, 1976; Menzel, 1980; Sevanian et al., 1982).

As shown in Figure 1, O_3 interacts with the ethylene group of PUFA by addition. The product decomposes to the Criegee zwitterion and an aldehyde, which recombine to form an ozonide (Roehm et al., 1971). The Criegee zwitterion can react with alcohols, carboxylic acids, or water to form peroxides. The peroxides can decompose to hydroxyl and alkoxyl radicals (Pryor et al., 1976), which can initiate the oxidation of membrane lipids (Menzel, 1976). In addition, the Criegee zwitterion can react with O_2 forming a ketone and regenerating O_3.

In addition to PUFA, O_3 can react with the thiol groups of proteins and peptides such as glutathione (Pryor et al., 1982). Oxidation of protein sulfhydryl groups can result in perturbation of protein structure and loss of enzymic activity.

Nitrogen dioxide initiates lipid peroxidation (Figure 2) via addition to the ethylene group of fatty acids (Menzel, 1980). The resultant fatty acid free radical reacts with O_2 to form a peroxyl radical which can abstract hydrogen from an unsaturated fatty acid, resulting in the propagation of an autoxidative chain reaction (Pryor and Lightsey, 1981).

Lipid peroxidation also can be initiated by endogenously produced oxygen free radicals. In the cell, molecular oxygen is reduced, both enzymatically and non-enzymatically (Figure 3), resulting in the formation of a variety of reactive oxygen compounds (Chance et al., 1979). Univalent reduction produces the superoxide anion radical ($O_2^{-}\cdot$) which can be reduced further either enzymatically, through the activity of superoxide dismutase, or nonenzymatically to H_2O_2. Hydrogen peroxide also is generated via divalent reduction of O_2 by a variety of membrane-bound enzymes (Chance et al., 1979). Hydrogen peroxide can react with O_2 in the presence of Fe^{+3} (Haber and Weis reaction) or Fe^{+2} (Fenton reaction) to yield the highly reactive hydroxyl radical ($\cdot OH$). In addition, H_2O_2 can react nonenzymatically with O_2 to form $\cdot OH$ or singlet oxygen ($^1O_2$), an extremely potent oxidant species. Singlet oxygen also can be generated via dismutation of $O_2^{-}\cdot$, especially at low pH.

Lipid peroxidation proceeds as illustrated in Figure 2. The peroxidative chain reaction is initiated by abstraction of a hydrogen from a

methylene group by a radical species producing a fatty acid radical which undergoes rearrangement to a conjugated diene radical (McCay and King, 1980). Such a radical can react with O_2 to form a peroxy radical which, in turn, can abstract a hydrogen from another PUFA molecule propagating the radical chain reaction (Figure 4). Alternatively, if the PUFA peroxy free radical contains three or more olefinic bonds, it can undergo cyclization to the corresponding monocyclic or bicyclic endoperoxide (Porter et al., 1976; Menzel, 1980). In the biosynthesis of prostaglandins, cyclooxygenase catalyzes production of PUFA peroxy free radicals (Olive et al., 1983) which subsequently undergo cyclization. The prostaglandin-like structure of certain of the cyclized oxidation products of PUFA suggests that they might function as prostaglandin analogs. The monocyclic compounds have been shown to inhibit arachidonic acid-induced aggregation of human platelets and the structure of the bicyclic compounds is analagous to that of the G and H series of prostaglandins (Porter et al., 1976). In addition, the physiological activity of certain prostaglandin endoperoxide intermediates is mimicked by fatty acid ozonides (Menzel, 1979).

In the lung, the major toxic effects of O_3 exposure are lipid peroxidation which can result in decreased activity of membrane bound enzymes and sulfhydryl oxidation which can result in decreased activity of sulfhydryl-requiring enzymes (Mustafa and Tierney, 1978). Vitamin E inhibits lipid peroxidation by sequestering free radicals (Fig. 4) and, in the lung, is consumed as a function of ozone exposure Indeed, the total tocopherol level of serum as well as that of other tissues is

decreased in ozone-exposed animals (Goldstein et al., 1970). However, although free radicals are not sufficiently stable to circulate from the lung to other tissues, it has been demonstrated that fatty acid ozonides injected intravenously into the rat can cause lung injury similar to that following ozone exposure (Cortesi and Privett, 1972). Thus, it appears reasonable to speculate that circulating fatty acid ozonides originating in the lung following ozone exposure can induce lipid peroxidation in nontarget tissues.

Evidence of lipid peroxidation as a mechanism of NO_2 toxicity includes the demonstration of : (i) increased amounts of lipid peroxidation products in the lung following NO_2 exposure (Sevanian et al., 1979; Sagai et al., 1982) and (ii) the behavior of NO_2 as a free radical in in vitro model systems (Roehm et al., 1971; Mead et al., 1980; Pryor and Lightsey, 1981). In addition, vitamin E deficiency has been reported to enhance NO_2-induced lipid peroxidation (Menzel et al., 1972; Fletcher and Tappel, 1973; Evans et al., 1980; Elsayed and Mustafa, 1982; Sevanian et al., 1982). The site of NO_2-induced injury is the epithelium of terminal and respiratory bronchioles (Stephens et al., 1972; Stephens et al., 1978). Such damage is followed by inflammatory processes which include the influx of macrophages and polymorphonuclear leukocytes (Gardner et al., 1969; DeNicola et al., 1981) and the proliferation and differentiation of cells for repair of the epithelium (Evans et al., 1973, 1975; Evans et al., 1976). To implicate lipid peroxidation as a primary mechanism of NO_2-induced lung damage, therefore, requires evidence of lipid peroxidation prior to the advent of the

inflammatory and repair processes (Guth and Mavis, 1986). Perusal of the literature indicates that measurements of lipid peroxidation products usually have been performed at the time of occurrence of the inflammatory and reparative responses and not prior to their advent. Therefore, the products of lipid peroxidation which have been measured may have derived mainly from peroxidative damage to dead cells and/or cellular debris (Stephens et al., 1972; Stephens et al., 1978) or the action of reactive species formed during phagocytosis (Shohet et al., 1974; Stossel et al., 1974; Kanner and Kinsella, 1983) instead of the direct action of NO_2 on lung tissue. This point must be considered in in vivo studies focused on investigation of the mechanism of oxidant-induced tissue injury.

Optimal cell/tissue functioning requires control of oxidative reactions associated with the production of energy, biosynthesis, defense against infection, and chemical toxicity, etc. Control is mediated through an antioxidant defense system, present in all cells comprised of oxidant scavenging molecules such as vitamin E (α-tocopherol), glutathione (GSH), ascorbic acid (AH_2), and uric acid (UA) and the enzymes superoxide dismutase, catalase, GSH peroxidase, GSH synthetase, GSH reductase, GSH transferase, and semidehydroascorbate reductase. Toxicants can upset the oxidant/antioxidant balance of cells by inhibiting enzymes, reacting with oxidant scavengers, or by forming free radical intermediates which initiate uncontrolled tissue reactions with molecular oxygen. Recent reviews have disucssed the various antioxidant systems present in lung tissue (Fridovich and Freeman, 1986; White and

Repine, 1985) and insights into antioxidant protective mechanisms (Ames, 1983; Burton et al., 1985).

COMPILATION OF ANTIOXIDANTS IN LUNG

A compilation of the concentrations of antioxidant scavenging molecules and enzymes within the rat lung (expressed per wet wt) is shown in Table I. GSH and ascorbic acid (AH_2) are considered the major water-soluble antioxidants and both have similar molar concentrations in the lung. α-Tocopherol is present in concentrations between 1/30 and 1/40 of the GSH and AH_2. The pyridine nucleotides are included in the table because they supply reducing equivalents for the regeneration of the common antioxidants. β-carotene, uric acid, and bilirubin have been hypothesized to be important protective agents (Ames, 1983; Stocker et al., 1987); however, there have been few studies reporting their concentrations in tissues. The enzymes appear to be present in high enough concentration to regenerate the entire cellular antioxidant pool within minutes of its oxidation. For comparison, the molar flux of ozone into the lung is given in the table at a concentration which produces edema (a detectable increase in lung wet weight) within 24 hr. An upper limit for the O_3 flux of approximately 0.06 μmoles/hr per gram of lung is expected based on the calculated resting breathing rate of rats (Stahl, 1967). If O_3 were to react stoichiometrically with α-tocopherol, all of the α-tocopherol of the lung parenchyma would be consumed within approximately an hour.

It has been reported that the vitamin E:PUFA ratio in biomembranes is of the order of 1:1000 (Fukuzawa et al., 1981). However, understanding of the integration of vitamin E into the structure of biomembranes is limited. Structural models suggest that, in the straight chain configuration, the polyunsaturated fatty acyl groups of the membrane phospholipids assume a quasi-helical conformation which contains "pockets." The "pockets" accomodate certain methyl groups of the phytol chain of vitamin E and stabilize the complex through London disperson and van der Waals forces (Lucy, 1972). For example, the "pockets" of the arachidonyl residues would interact with the C'4 and C'8 methyl groups of the phytol side chain. At least two methylene-interrupted cis double bonds are required for "pocket" formation. Three or more methylene-interrupted double bonds provide for optimal interaction.

PULMONARY ANTIOXIDANT ENZYMES

In the lung, as in other organs, specific enzymes are responsible for protecting the cell against oxidants and reactive oxygen species. Among these enzymes are catalase (CAT), glutathione peroxidase (GSH Px), and superoxide dismutase (SOD), which act in concert in the cell.

It has been reported that, following ozone exposure, the apparent activity of lung GSH-Px, glucose-6-phosphate dehydrogenase (G-6-PD) and GSH reductase is increased (Chow and Tappel, 1973; Plopper et al., 1979), and, for a variety of biochemical parameters, the effects of intermittent exposure do not differ significantly from those of continuous exposure (Mustafa and Tierney, 1978).

In addition to oxidative insult from exogenous sources, oxidant species are generated endogenously. For example, aerobically respiring cells produce H_2O_2 via divalent reduction of O_2 and the dismutation of the superoxide anion radical ($O_2^{\cdot-}$). The reduction of H_2O_2 to H_2O is catalyzed by catalase and peroxidase.

Catalase

Catalase, which has a high k_m (~ 1.1M) for H_2O_2 (Ogura, 1955), is located primarily in the peroxisomes. Peroxisomes appear to be associated with smooth endoplasmic reticulum and have been shown to be localized in pulmonary Type II epithelial cells, bronchiolar Clara cells, and alveolar macrophages (Goldenberg et al., 1978; Davies et al., 1979). Catalase activity commonly is measured by the UV assay of Aebi (1974) in which the decrease in extinction at 240nm resulting from the decomposition of H_2O_2 is measured. Since accurate assay of tissue levels of the enzyme requires complete lysis of peroxisomes, it is necessary to add a detergent, such as 1% Triton X-100, to the tissue homogenate.

Glutathione Peroxidase (GSH Px)

Glutathione peroxidase was first discovered in red blood cells where it inhibits the oxidative decomposition of hemoglobin (Mills, 1957). Glutathione peroxidase exists in two forms: a selenium-dependent tetrameric enzyme (Se-GSH-Px) with a M_r of approximately 84,000 and a selenium-independent dimeric enzyme (non-Se-GSH-Px) with a M_r of approximately 45,000. Both types have been isolated and purified from

rat lung (Lawrence and Burk, 1978; Jenkinson et al., 1983), as well as other tissues of several species (Lawrence and Burk, 1978). The non-Se-GSH-Px catalyzes degradation of organic, but not inorganic peroxides (Lawrence and Burk, 1976). The Se-GSH-Px is located in the cytosol and mitochondrial matrix and has a k_m for H_2O_2 of 1.0 μM (Flóhe and Brad, 1969). The enzyme contains four gram atoms of Se per mole (~ 84K), and Se is essential for catalytic activity (Flóhe et al., 1973; Rotruck et al., 1973). In addition to H_2O_2, Se-GSH-Px catalyzes the reduction of organic hydroperoxides to their corresponding alcohols (Christopherson, 1968; Little and O'Brien, 1968).

On a wet weight basis, the Se-GSH-Px activity of the human lung is approximately 30% lower than that of the rat lung (Jenkinson et al., 1984). The human lung also exhibits non-Se-GSH-Px activity (Lawrence and Burk, 1978). The Se-independent enzyme comprises approximately 34% of the total GSH-Px activity of the rat lung, the remainder being contributed by the Se-dependent enzyme (Jenkinson et al., 1983). The non-Se-GSH-Px activity is associated with glutathione-S-transferases (GSH-S-Tr), a group of multifunctional enzymes involved in the biotransformation/detoxification of a broad spectrum of xenobiotic and endogenous compounds (Prohaska and Ganther, 1977; Lawrence et al., 1978). The GSH-S-Trs of rat liver (Reddy et al., 1981) and rat lung (Jenkinson, et al., 1983) contain significant non-Se-GSH-Px activity, and it has been observed that non-Se-GSH-Px inhibits microsomal lipid peroxidation (Lawrence et al., 1978).

Total GSH-Px activity usually is measured by monitoring the oxidation of NADPH at 340 nm by a modification of the coupled enzyme procedure (Figure 4) with cumene hydroperoxide as substrate (Paglia and Valentine, 1967). Since the assay is coupled to GSH reductase, conditions affecting the activity of true reductase impinge on the accuracy of the assay. Under such conditions, the direct spectrophotometric method of Splittgerber and Tappel (1979) may be employed to assay GSH-Px activity. One unit of GSH-Px activity is defined as the amount of enzyme catalyzing the oxidation of 1 µmol of NADPH per minute at 25°C in the coupled assay system and as a change of 1 absorbance unit/minute at 25°C in the direct spectrophotometric assay.

The contribution of non-Se-GSH-Px to total GSH-Px activity is determined by subtraction. Total GSH-Px activity is determined with cumene hydroperoxide as substrate. Se-GSH-Px activity is measured using H_2O_2 as substrate. Since catalase also catalyzes the reduction of H_2O_2, it is necessary to inhibit catalase activity with azide. Non-Se-GSH-Px activity is estimated by subtracting the difference between total GSH Px activity and that contributed by Se-GSH-Px. This assay has been adapted to the centrifugal analyzer (Centrifichem 400, Baker Instruments) for automated analysis of animal tissues (Jaskot et al., 1983), which allows for the processing of large numbers of samples.

The reduced glutathione (GSH)/oxidized glutathione (GSSG) couple is the largest mobile thiol redox system of the cell (Chance et al., 1979). The cellular level of GSH is maintained by the reduction of GSSG catalyzed by the flavin enzyme, glutathione reductase (Buzard and Kopko,

1963), and the de vovo synthesis of GSH (Chow, 1979). The reduction of GSSG requires reducing equivalents in the form of NADPH. The cellular NADPH level is maintained by two enzymes of the hexose monophosphate pathway glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (Figure 4).

The enzymes apparently function complementarily in protecting the cell against lipid peroxidation (Hochstein and Utley, 1968; Sunde and Hoekstra, 1980). For example, lipid hydroperoxides readily undergo metal-catalyzed homolytic cleavage to reactive peroxyl (LOO·), alkoxyl (LO·), and hydroxyl radicals, which can initiate/propagate lipid peroxidation. Hydroxyl radical also is generated by the interaction of $O_2^{-}\cdot$ and H_2O_2 (Haber-Weis reaction), the Fenton reaction (Figure 3), and metal catalyzed interaction of $O_2^{-}\cdot$ and H_2O_2, which also generates singlet oxygen ($^1O_2$). However, the role of Se-GSH-Px in the reduction of membrane-bound lipid hydroperoxides has been questioned (McCay et al., 1981). It was observed (McCay et al., 1976) that Se-GSH-Px can reduce fatty acid hydroperoxides, but not membrane-bound lipid hydroperoxides, suggesting that release of fatty acid hydroperoxides from biomembranes (by the action of phospholipase) is prerequisite for effective functioning of the enzyme. Also, a GSH heat-labile factor which is not Se-GSH-Px has been described which functions to inhibit lipid peroxidation (McCay et al., 1981).

Catalase and peroxidase function to control the cellular level of H_2O_2 (Oshino and Chance, 1977; Chance et al., 1979) and regulate the hydroperoxide tone of the cell (Hemler et al., 1979). The level of

cellular peroxidase activity appears to be maintained by a balanced contribution of the various enzymes utilizing peroxides as substrate. If the activity of any one of the enzymes is decreased significantly, for example, Se-GSH-Px in selenium deficiency, the apparent activity of other enzymes of the group is elevated to compensate for the deficiency (Serfass and Ganther, 1976; Bryant and Bailey, 1980). Thus, Burk et al. (1978) observed a marked increase in liver catalase activity in selenium-deficient rats in apparent compensation for the loss of selenium-dependent H_2O_2 removal capacity. Also, in apparent response to selenium deficiency, non-Se-GSH-Px activity has been reported to be increased (Lawrence et al., 1978; Stone and Dratz, 1980). The hydroperoxide tone of the cell may play an important role in the regulation of enzymes involved in the biosynthesis of prostaglandins, thromboxanes, and leukotrienes (Samuelsson, 1981).

Superoxide Dismutase (SOD)

Superoxide dismutase catalyzes the reduction of the superoxide anion radical via dismutation (Figure 3) (Fridovich,1976; Fridovich,1979). This appears to be the only physiological function of the enzyme. The enzyme is found in all oxygen-consuming organisms (McCord et al., 1971) and is a metalloprotein. Iron-dependent (Fe-SOD) and manganese-dependent (Mn-SOD) forms of the enzyme are found in prokaryotes. Copper/zinc-SOD (Cu/Zn-SOD) is found in the cytosol and Mn-SOD in the mitochondrial matrix of eukaryotic cells. In the rat lung, the mitochondrial enzyme exhibits approximately 1/5 the activity of the cytosolic enzyme on a unit/mg DNA basis (Forman and Fisher, 1981). The

Cu/Zn-SOD and Mn-SOD of the human lung have been isolated (Marklund, 1982 and 1984a). A third isoenzyme, extracellular-SOD (EC-SOD), also has been isolated and purified from human lung (Marklund, 1984b). EC-SOD is a ~ 135,000-dalton tetrameric, hydrophobic glycoprotein, which contains 4 copper atoms and 4 zinc atoms (Marklund, 1982). EC-SOD differs from Cu/Zn-SOD in antigenic properties (Marklund, 1984b) and amino acid composition (Marklund, 1982). The physiological role of EC-SOD is unclear. However, EC-SOD has been shown to be the major SOD in human extracellular fluids (Marklund et al., 1982) and accounts for approximately 5% of lung SOD activity as determined by immunological antibody separation.

Direct measurement of SOD activity requires special equipment (Ballou et al., 1969; Rotilio et al., 1972). Therefore, indirect measurement based on SOD inhibition of an $O_2^{\cdot -}$ -induced reaction generally is employed (Paoletti et al., 1986). $O_2^{\cdot -}$ can be generated either enzymatically or non-enzymatically (McCord et al., 1977; Flóhe and Otting, 1984). SOD activity can be detected by colorimetric (Crapo et al, 1978) luminometric (Bensinger and Johnson, 1981) or polarographic methods (Rigo et al., 1975). A method of general utility is that of Crapo et al. (1978), which is based on the ability of SOD to inhibit the rate of reduction of cytochrome c in the presence of xanthine and xanthine oxidase (Figure 3). Briefly, cytochrome c reduction is measured after 30 sec of incubation at 25°C with xanthine and xanthine oxidase in potassium phosphate buffer (pH 10) in the absence (control) and presence of SOD. The magnitude of the decrease in absorption

recorded at 550 nm is the measure of SOD activity. At pH 10, non-specific interference by cytochrome oxidase, which occurs at lower pH, is eliminated. Also at pH 10, the assay is approximately 10 times more sensitive for the Cu/Zn SOD than the Mn-SOD. Analysis at pH 10 and pH 7.8 can be used to distinquish between the Cu/Zn and Mn enzymes.

Other enzymes, for example semidehydroascorbate reductase, GSH reductase, GSH synthetase, glucose-6-phosphate dehydrogenase, and GSH transferase may function directly or indirectly in the removal of potentially toxic forms of oxygen, but will not be addressed in this chapter.

ANTIOXIDANT SUBSTANCES

Vitamin E (α-tocopherol)

α-Tocopherol, which blocks propagation of lipid peroxidation by scavaging PUFA peroxy free radicals (Wilson *et al.*, 1978), (Figure 4), is highly lipophilic and is integrated into biomembranes (Lucy, 1972). Such integration is requisite for effective PUFA free radical chain-terminating activity (Tinberg and Barber, 1970) and an increased rate of lipid peroxidation is observed in vitamin E deficient animals (Tappel, 1962).

Recent symposia have reviewed the chemistry and physiology of vitamin C or ascorbic acid (AH_2) (Counsel and Hornig, 1981; Seib and Tolbert, 1982) and GSH (Larsson *et al.*, 1983; Meister and Anderson, 1983). Recently, uric acid (UA) has been suggested to be an important water-soluble antioxidant (Ames, 1983). The lung concentrations of AH_2,

GSH, and α-tocopherol in human cancer surgery patients along with those of 5 laboratory animal species as well as sheep and swine have been compared (Slade *et al.*, 1985). Little information is available on the relative concentrations of the antioxidants in different cell types. The presence of AH_2 (Skoza *et al.*, 1983) and GSH (Sutherland *et al.*, 1985) has been reported in the surfactant lining of the lung. However, it has not been substantiated that extracellular antioxidants play an important role in the antioxidant defense mechanism of the lung.

Three types of studies have been employed to investigate the effects of oxidant exposure on water-soluble antioxidant molecules: (1) the antioxidants are exposed directly to gaseous oxidants *in vitro* and their oxidation/degradation is observed; (2) animals or cells are exposed to a gaseous oxidant and the effects on the concentrations of their constituent antioxidants are determined; (3) the concentrations of antioxidants in animal tissue or cells in culture are altered by diet or drug treatment and the effect of such alteration on oxidant toxicity is observed.

In vitro exposures of antioxidants to gaseous oxidants have been studied. Mudd and coworkers (1969) demonstrated the oxidation of cysteine and other amino acids by O_3 *in vitro*. Likewise, Menzel (1971) showed that glutathione, NADH and NADPH are oxidized by O_3. Further studies of this nature may be useful in determining which antioxidants are most critically affected by specific oxidants and conditions of exposure. One factor to be considered in such studies is whether the concentrations of oxidant and antioxidant *in vitro* are comparable to those found *in vivo*. It may also be important to use a mixture of

antioxidants rather than single compounds because of possible interrelationships between the antioxidants. For example, AH_2 or GSH alone or in combination with other antioxidants may be responsible for reducing α-tocopherol after its oxidation (Tappel, 1962; Leung et al., 1981; Reddy et al., 1982). Changes induced in tissue levels of one (or more) antioxidant have been reported to affect the levels of others (Chen and Chang, 1979). Significant correlations among lung levels of the antioxidants have been observed in laboratory animals (Slade, et al., 1985).

Concentrations of water-soluble antioxidants often are decreased acutely (within hours) in lungs following inhalation exposures to oxidants severe enough to cause increased lung wet weight (Leung and Morrow, 1981; DeLucia et al., 1975; Arad et al., 1980).

Following exposure, the lung antioxidants often rebound to higher than normal levels (Dodd et al., 1979; Chow et al., 1976; Dubick et al., 1985; Hatch et al., 1986). Since the magnitude of this increase in antioxidants appears to be greater than the increased lung weight changes which accompany edema and repair, it does not appear to be the simple result of the changing tissue weight. The increase in GSH probably is the result of increased synthesis since it appears to be partially inhibited by buthionine sulfoximine (BSO), a specific inhibitor of γ-glutamylcysteine synthetase (Griffith and Meister, 1979b). Impairment of tissue repair may be another consequence of interference with the GSH increase (Sun et al., 1986).

In rats and mice, lung AH_2 levels have been shown to increase following oxidant exposures (Dubick et al., 1985; Chow et al., 1976).

AH_2-deficient guinea pigs exposed to NO_2 also show increases in both AH_2 and non-protein sulfhydryls (Hatch et al., 1986). Because no synthesis of AH_2 occurs in guinea pigs, this increase is probably the result of increased uptake from other tissues or decreased excretion. Factors which control the AH_2 concentrations in cells are not well understood. Dubick et al. (1983) reported increased AH_2 turnover in the lungs of mice following O_3 exposure. In most tissues, AH_2 transport into cells is a Na^+-dependent, energy-requiring process (Rose, 1981; Rose, 1986). Intestinal active transport of AH_2 is present in those species requiring the vitain, but only passive diffusion occurs in those species that synthesize the vitamin (Stevenson and Brush, 1969; Rose, 1981). Perfused lungs appear to take up AH_2 by a carrier-mediated process (Willis and Kratzing, 1975). In isolated type II cells and alveolar macrophages, the influx of AH_2 is Na^+-dependent and linked to cellular metabolism (Castranova et al., 1983).

GSH and to a lesser extent GSSG appear to be transported out of cells (Meister, 1983). This transport appears to be the major reason GSH levels are decreased when its synthesis is inhibited by BSO administration (Griffith and Meister, 1979a). Transport into cells appears to be controlled by a membrane γ-glutamyl transpeptidase which appears to be present only in selected tissues (Griffith and Meister, 1979a; Anderson et al., 1985). This enzyme catalyzes the breakdown of GSH to γ-glutamyl peptides that are actively transported into the cells. Although the lung possesses γ-glutamyl transpeptidase, little information on GSH transport mechanisms and rates of turnover of GSH in lung tissue is available (Anderson et al., 1985).

In vivo studies in which the concentrations of antioxidant tissue are selectively altered can provide information on the function of particular antioxidants under conditions of oxidant stress. Recently, Meister and coworkers have reported methods for selectively altering tissue GSH concentrations employing BSO or GSH esters (Griffith and Meister, 1979b; Anderson *et al.*, 1985).

In guinea pigs, reduction of lung AH_2 concentrations can be easily obtained using rabbit chow (not supplemented with AH_2), and compensating for the lack of complete definition of the diet by assaying the lung tissue for all major antioxidants (Hatch *et al.*, 1986). It has been observed that AH_2 deficiency enhances the toxicity of inhaled NO_2 (Selgrade *et al.*) but has little effect on the toxicity of inhaled O_3 (Hu *et al.*, 1982).

Assay Methods for Tissue Antioxidants

Extraction of water-soluble antioxidants (AH_2, uric acid, GSH) from tissues for assay can be achieved by homogenizing the tissue in at least 8 volumes of 3% perchloric acid. Following centrifugation at 20,000 g, the supernatant can be stored at -70°C.

Spectrophotometric, colorimetric, and fluorometric methods are available for the assay of AH_2 (Bliss and György, 1967; Omaye *et al.*, 1979; Sauberlich *et al.*, 1982). Non-chromatographic methods may be subject to interference from substances present in tissue extracts. Liquid chromatographic (LC) techniques employing reverse phase (Wagner *et al.*, 1979; Vandemark and Schmidt, 1981; Iriyama *et al.*, 1984), anion exchange

(Rose and Nahrwold, 1982; Carr et al., 1983; Hatch and Sevanian, 1984), or paired-ion reverse phase systems (Finley and Duang, 1981; Tsao and Salimi, 1982; Keating and Haddad, 1982; Kutnink et al., 1985) have been developed to assay AH_2. Some of these methods also can be used to simultaneously assay UA (Aoki et al., 1984; Hatch and Sevanian, 1984; Iriyama et al., 1984; Kutnink et al., 1985). Electrochemical detection appears to provide greater sensitivity and selectivity since only oxidizable substances are measured.

Colorimetric, fluorometric and enzymatic methods have been developed for assaying GSH in biological samples (Meister and Anderson, 1983; Anderson, 1985). Colorimetric methods have detection limits in the range of 100-200 ng (Sedlak and Lindsay, 1968; Prins and Loos, 1969), while the limit for fluorometric methods is about 10 ng (Hissin and Hilf, 1976; Keller and Menzel, 1985).

Tissue levels of α-tocopherol can be quantified by colorimetric (Emmerie and Engel, 1939; Tsén, 1961) or spectrofluorometric (Duggan, 1959; Taylor et al., 1976) methods, thin layer chromatography (Bieri and Prival, 1965), gas-liquid chromatography (Sheppard et al., 1972; Lehman, 1978) or high performance liquid chromatography (HPLC) using ultraviolet absorption, fluorometric detection (Buttress and Diplock, 1984) or electrochemical detection (Vandewoude et al., 1984; Chow et al., 1985). Quantitative extraction of α-tocopherol from tissues can be accomplished with a variety of solvents (Burton et al., 1985).

SUMMARY

In summary, it appears that oxidation reactions are key factors which probably contribute significantly to the toxicity of a number of inhaled pollutants. Antioxidant substances and enzymes that are normally present in tissues of the respiratory tract appear to function in a protective role against pathological oxidation. Further research is needed to better define tissue differences in the concentrations of antioxidants and to determine how these differences might affect the toxicity of inhaled compounds.

TABLE 1

BALANCE BETWEEN OXIDANTS AND ANTIOXIDANTS IN RAT LUNG

BASED ON OZONE EXPOSURE LEVELS AND KNOWN ANTIOXIDANT CONCENTRATIONS

	Lung Oxidant/Antioxidant Concentration or Activity	Reference
Antioxidants		
α-Tocopherol	0.05 μmoles/g wet wt	Slade et al., 1985
Ascorbic acid	1.93 μmoles/g wet wt	Slade et al., 1985
Glutathione	1.56 μmoles/g wet wt	Slade et al., 1985
NADH	58.5 μmoles/g wet wt†	Dixon and Webb, 1964
NADPH	29.0 μmoles/g wet wt†	Witschi et al., 1977
β-carotene	0.65 nmol/g wet wt	Shapiro et al., 1984
Retinyl palmitate	0.15 μmol/g wet wt	Bhat and Lacroix, 1983
Enzymes		
Superoxide Dismutase	65 μmoles O_2^-/hr/g wet wt††	Frank et al., 1987
Semidehydroascorbate Reductase	53 μmoles Ascorbate/hr/g wet wt††	Diliberto et al., 1982
GSH-Reductase	108 μmoles NADPH/hr/g wet wt§	Mustafa et al., 1977
GSH-Peroxidase	120 μmoles NADP/hr/g wet wt§	Mustafa et al., 1977
G6P-Dehydrogenase	132 μmoles NADPH/hr/g wet wt§	Mustafa et al., 1977
Catalase	21,800 μmoles H_2O_2/hr/g wet wt§	Frank et al., 1978
Prototype Oxidant		
Inhaled Ozone, 1.0 ppm	0.06 μmoles O_3/hr/g wet wt	

†Assumes 6-8 μg DNA/g wet wt
††Assumes 100 mg protein/g wet wt
§Assumes a rat lung wet wt of 1.0 g

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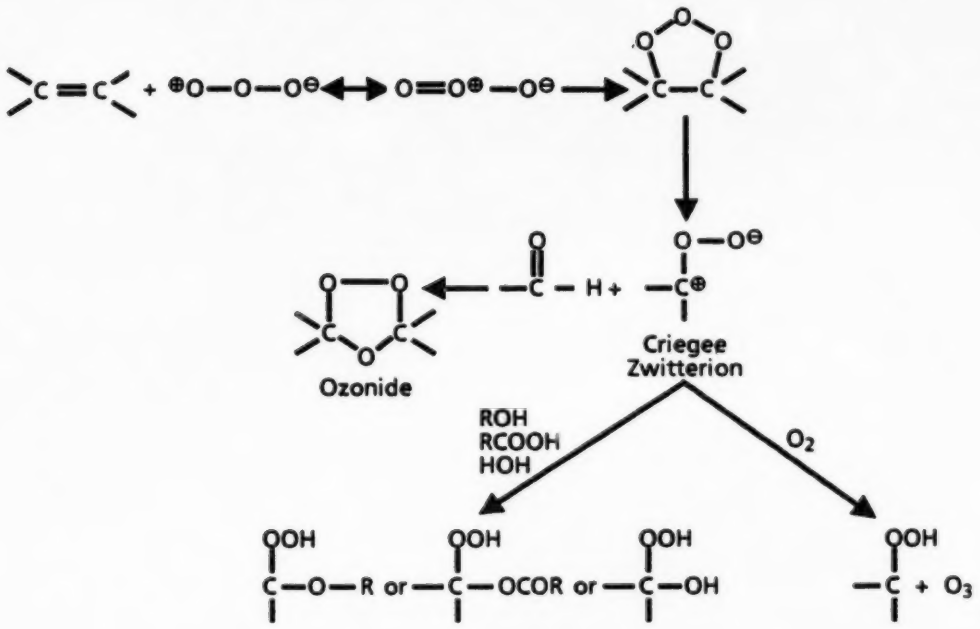

Figure 1. Oxidation of Olefins by Ozone

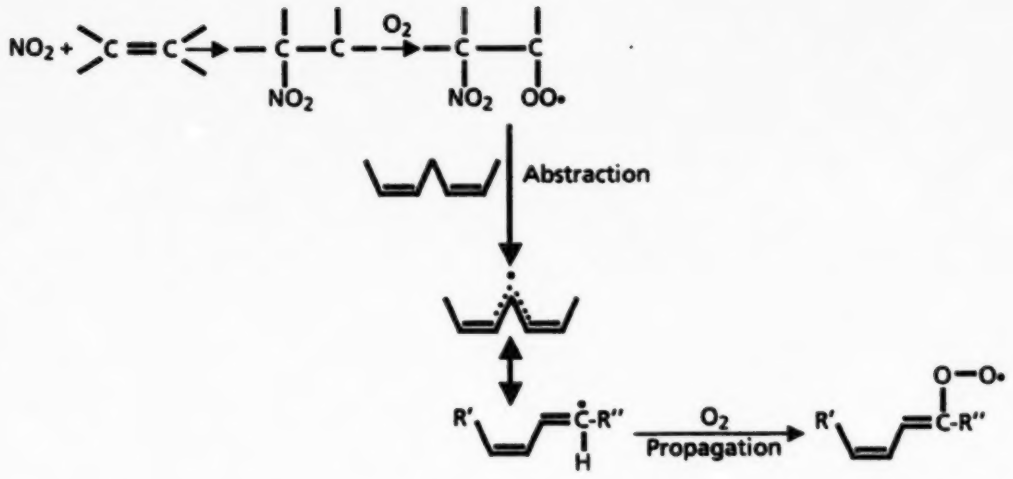

Figure 2. Oxidation of Olefins by Nitrogen Dioxide

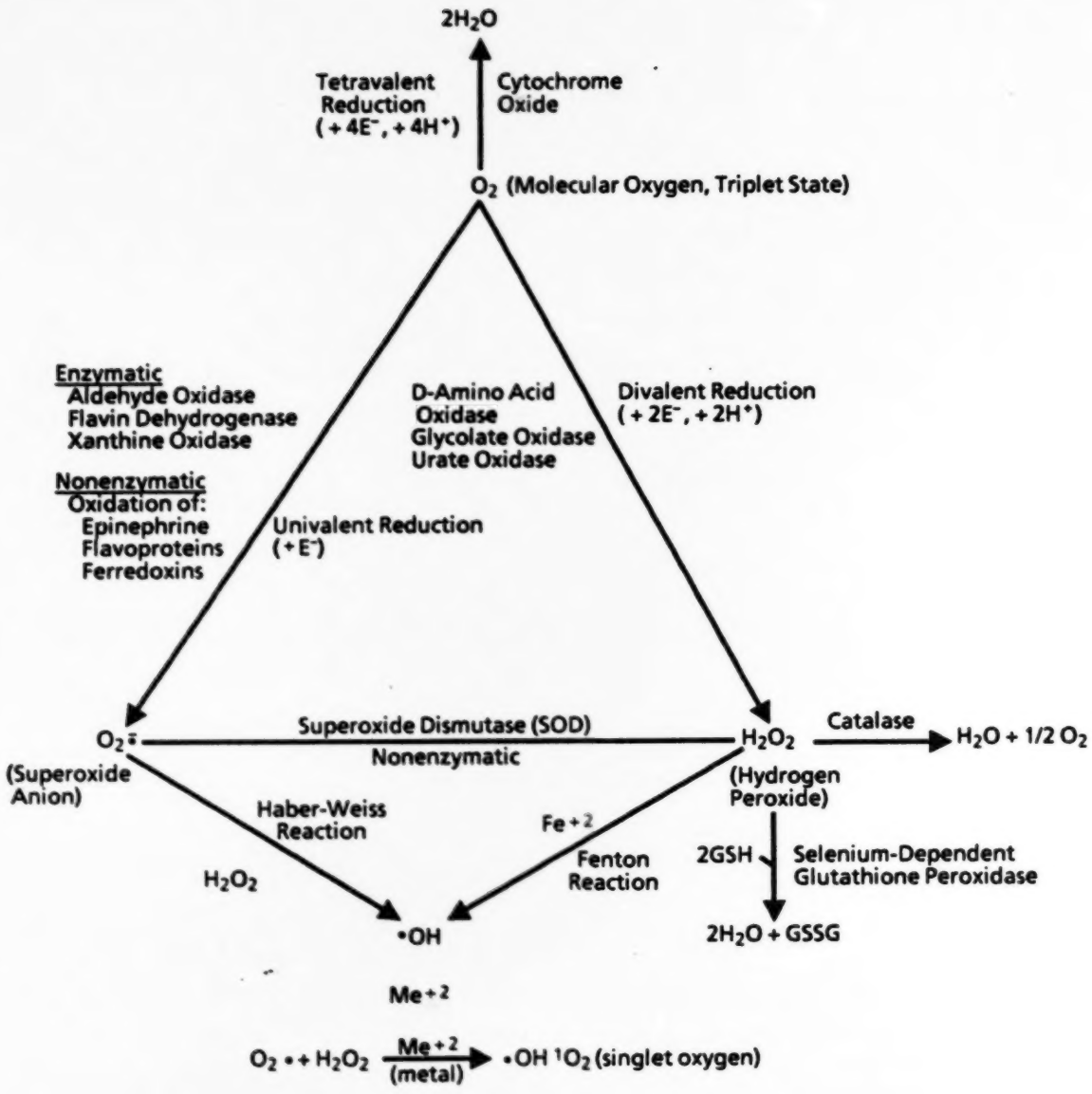

Figure 3. Cellular Production of Reactive Oxygen Intermediates

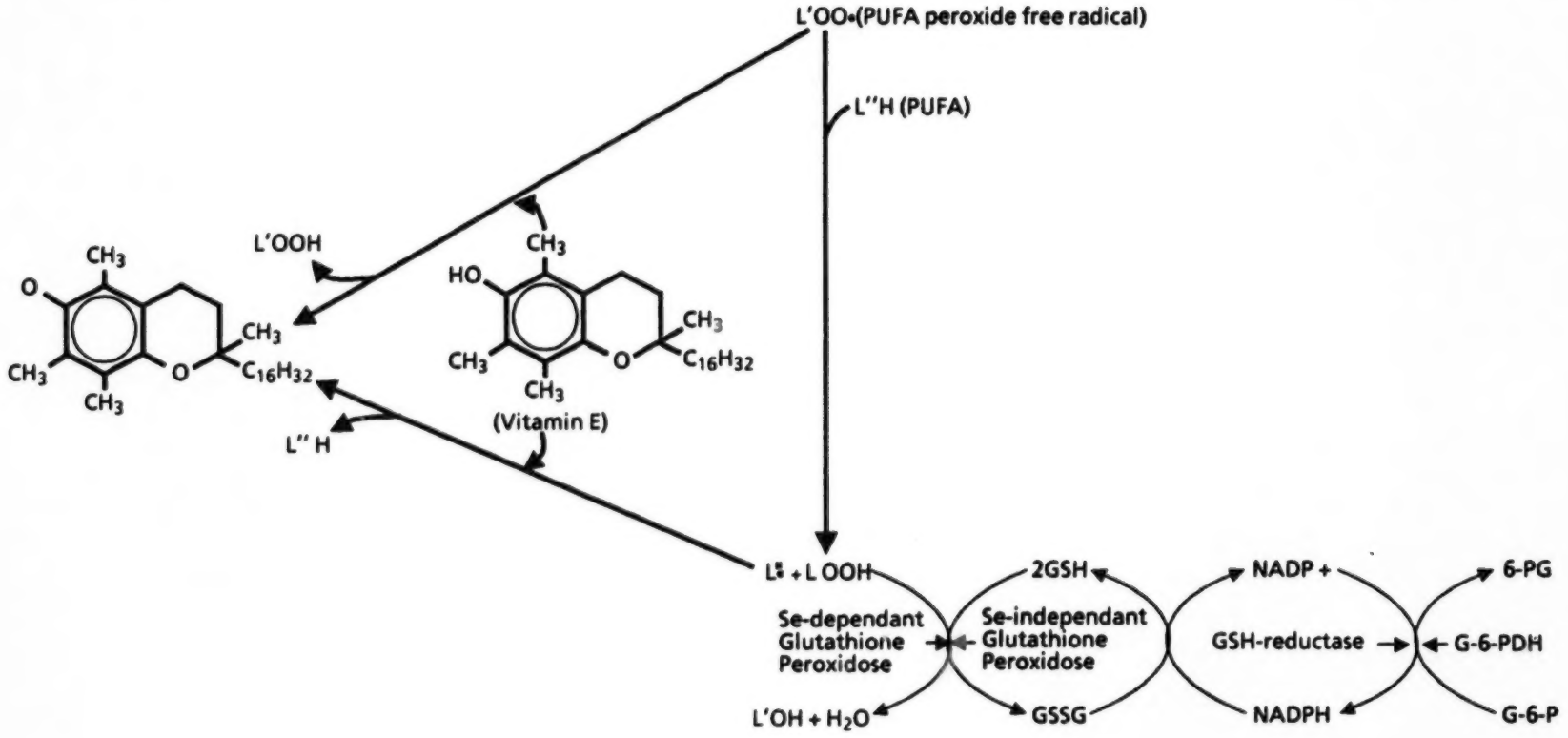

Figure 4. Control of PUFA Oxidation.